Antimicrobial Coumarin Derivative from *Delonix regia*

Consolacion Y. Ragasa and Joy G. Hofilena
Chemistry Department, De La Salle University, 2401 Taft Avenue, Manila

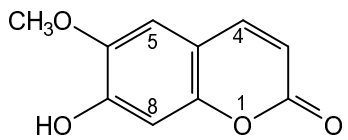
The dichloromethane extract of the air-dried leaves of *Delonix regia* afforded scopoletin (**1**) by silica gel chromatography. The structure of **1** was determined by spectroscopic methods and confirmed by comparison of its ^{1}H NMR data with those reported in the literature for scopoletin. Compound **1** exhibited antifungal activity against *Candida albicans* and antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus,* and *Bubtilis subtilis.* It was inactive against the fungi, *Aspergillus niger and Trichophyton mentagrophytes.*

Keywords*: Delonix regia, Leguminosae, scopoletin, antimicrobial*

1. INTRODUCTION

Delonix regia, also known as flame tree is widely cultivated adorning avenues and parks in the Philippines. The essential oil from the leaves of the plant is fungitoxic (Pandey, Chandra, & Tripathi, 1982). The aqueous extract of the bark has emetic effects in cats and monkeys (Pant & Joshi, 1972), while the aqueous flower extracts are active against the roundworm *Haemondus contortus* (Gupta & Chandra, 1971). A recent study reported that the metabolite-rich fractions of the sequentially extracted flowers and seeds of *D. regia* exhibited antifungal activity against *Aspergillus niger, Aspergillus flavus, Rhizopus bataticola* and *Fusarium auxisporum* (Sharma, R. A., Chandrawat, P., Sharma, S., Sharma, D., Sharma, B., & Singh, D., 2010). Earlier studies reported the isolation of flavonoids and flavonoid glycosides with antioxidant properties from the flowers (Su & Fan, 2008); Gupta & Chandra, 1971). β-sitosterol, lupeol, and their acetates (Roy & Sengupta, 1968), and carotenoids (Barua & Barua, 1966) were also isolated from the plant. A recent study reported the isolation of lupeol, epilupeol, β-sitosterol, stigmasterol and p-methoxybenzaldehyde from the stem bark of *D. regia* (Jahan et al., 2010).

We now report the isolation and antimicrobial test results of scopoletin (**1**) from the dichloromethane extract of the leaves of *D. regia.* To the best of our knowledge this is the first reported isolation of scopoletin from *D. regia.*



1

2. MATERIALS AND METHODS

2.1. General Experimental Procedure

The NMR spectra were measured on a Bruker Avance 400 in $CDCl_3$ at 400 MHz for ^{1}H. Column chromatography was performed

with silica gel 60 (70-230 mesh). Thin layer chromatography (TLC) was performed on plastic backed plates coated with silica gel F_{254}. TLC plates were visualized by spraying with vanillin-H_2SO_4, then warming.

2.2. Sample Collection

The leaves of the plant material were collected from San Pedro, Laguna in January 2001. It was identified as *Delonix regia* (Bojer) Raf. at the Philippine National Museum.

2.3. Isolation

The air-dried leaves (1.2 kg) of *D. regia* was soaked with dichloromethane for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (91 g) which was treated with 4% aqueous $Pb(OAc)_2$ to precipitate the more polar components (Padolina et al., 1974). The treated extract (4.5 g) was fractionated by silica gel chromatography and eluted with acetone in dichloromethane via gradient elution at 10% increments. The fraction eluted with 20% acetone in dichloromethane was rechromatographed (6×) in diethyl ether: acetonitrile: dichloromethane (1:1:8) to afford **1** (colorless solid, m.p. 200-202°C, 8 mg).

2.3.1. Antimicrobial Tests

The microorganisms used in these tests were obtained from the University of the Philippines Culture Collection (UPCC). These are *Pseudomonas aeruginosa* UPCC 1244, *Bacillus subtilis* UPCC 1149, *Escherichia coli* UPCC 1195, *Staphylococcus aureus* UPCC 1143, *Candida albicans* UPCC 2168, *Trichophyton mentagrophytes* UPCC 4193 and *Aspergillus niger* UPCC 3701. Compound **1** was tested for antimicrobial activity against these microorganisms.

Microbial suspensions were prepared from 24-hours-old cultures of the bacteria and yeast and from 5-days-old culture of the molds. The suspending medium used was 0.1 % peptone water. Pre-poured agar plates, about 3 mm thick, were inoculated with the microbial suspension by swabbing the agar surface. Nutrient agar (NA), Glucose Yeast Peptone (GYP) Agar and Potato Dextrose Agar (PDA) were used for bacteria, yeast, and molds, respectively. The cotton swab on an applicator stick was dipped into the microbial suspension, rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculums from the swab. The swab was streaked over the entire agar surface. This procedure was repeated two more times, rotating the plate 60^0 each time to ensure even distribution of the inoculums. Three equidistant wells were made on the agar plate using a cork borer (10 mm) and 200 μL of **1** dissolved in 95 % ethanol was placed in each hole.

The plates were incubated at room temperature. NA and GYP plates were observed after 24-48 hours and PDA plates were observed after 2-5 days. The clearing zone was measured in millimeters and the average diameter of the clearing zones was calculated. The activity index was computed by subtracting the diameter of the well from the diameter of the clearing zone divided by the diameter of the well

3. RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the air-dried leaves of *Delonix regia* afforded **1**. The structure of **1** was deduced by spectroscopic analyses as follows.

The uv spectrum of **1** in MeOH gave three λ_{max} at 353.5, 323, and 296 nm indicating a conjugated system. The IR spectrum indicated a hydroxyl (IR ν_{max} 3335 and 1118 cm^{-1}), a carbonyl of a lactone (1760 and 1294 cm^{-1}), and an aromatic C=C stretch (1567 cm^{-1}). The ^1H NMR spectrum of **1** (Table 1) showed resonances for ortho coupled aromatic protons (J = 9 Hz) at δ 6.26 and 7.59; two aromatic proton singlets at δ 6.84 and, 6.92 located para to each other, a broad singlet of a hydroxyl proton at δ 6.14; and methoxy protons at δ 3.96

(3H, s). These spectroscopic data are typical for a coumarin derivative with a methoxy and a hydroxyl substituents (Juan, Rideout, & Ragasa, 1997).

Table 1.
Comparison of the 400 MHz ^{1}H NMR Spectral Data of **1** *and 400 MHz ^{1}H NMR Spectral Data of scopoletin in $CDCl_3$ and the NOESY Spectral Data of 1 in $CDCl_3$*

Position	δ_H mult. (*J* Hz)	NOESY Correlations	δ_H mult. (*J* Hz) scopoletin (Siddiqui et al., 2007)
1	---	---	
2	---	---	
3	6.26 (d, 9 Hz)	H-4	6.26 (d, 9.4 Hz)
4	7.59 (d, 9 Hz)	H-3, H-5	7.58 (d, 9.4 Hz)
4a	---	---	
5	6.84 (s)	H-4, 6-OCH_3	6.89 (s)
6	---		
7	---		
8	6.82 (s)		6.82 (s)
8a	---		
6-OCH_3	3.96 (s)	H-5	3.93 (s)
7-OH	6.14 (br s)		

The positions of the substituents were determined from the NOESY spectrum (Table 1 and Figure 1) which indicated that H-3 was close in space to H-4, which was in turn close to H-5, which was finally close to the C-6 methoxy. The H-8 and H-5 were located para to each other since they were both singlet. Literature search revealed that **1** is scopoletin as evidenced by similar ^{1}H NMR data (Siddiqui, B. S., Sattar, F. A., Ahmad, F., & Begum, S., 2007). Furthermore, the melting point of **1** is identical to the melting point of scopoletin (Crosby, 1961). A comparison of the ^{1}H NMR data of **1** and scopoletin is shown in Table 1.

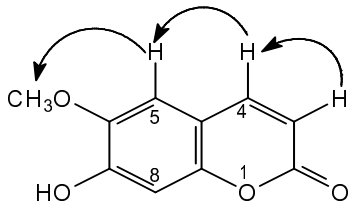


Figure 1. Key NOESY correlations of **1**

A current study reported that scopoletin isolated from *Protium javanicum* exhibited the strongest termiticidal activity among the 10 compounds tested (Adfa, Yoshimura, Komura, & Koketsu, 2010). The memory impairments in Alzeimer's disease result from a deficit of cholinergic functions of the brain. A therapeutic strategy has been the use of inhibitors of acetylcholinesterase (AChE). Scopoletin inhibited the AChE activity in a dose-dependent manner and its IC_{50} value was 10.0 μg/mL (Lee, H. L., Lee, K. T., Yang, Baek, & Kim, 2004). The chloroform soluble fraction of a methanol extract of *Phyllanthus reticulates* showed strongest cytotoxicity to brine shrimp lethality bioassay with LC_{50} of 1.99 μg/mL. Scopoletin was isolated from this fraction (Begum, Rahman, & Rashid, 2006). In another study, the chloroform extract of *Nepheleum longan* stem bark showed promising results in the brine shrimp lethality assay. The extract also exhibited antifungal and antibacterial activities. Purification of the extract afforded scopoletin and stigmasterol (Rahman, Nahar, Khan, & Hasan, 2007).

As part of our continuing search for antimicrobial compounds from Philippine medicinal plants and since *D. regia* is known to exhibit antifungal activity, **1** was tested for possible antimicrobial activity against the gram positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*; gram negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli*; and fungi, *Candida albicans, Trichophyton mentagrophytes*, and *Aspergillus niger*. Results of the tests (Table 2) showed that **1** is active against *C. albicans* with an activity index (AI) of 0.3 and slighty active against *P. aeruginosa, E. coli, S. aureus* and *B. subtilis* with AI of 0.2. It was inactive against *A. niger* and *T. mentagrophytes.*

Table 2.
Antimicrobial Test Results on **1.**

Organism	Sample[a] (30 μg)	Clearing Zone, mm*	Activity Index (A.I.)
Escherichia coli	**1**	12	0.2
	Chloramphenicol	24	1.4
Pseudomonas aeruginosa	**1**	12	0.2
	Chloramphenicol	12	0.2
Staphylococcus aureus	**1**	12	0.2
	Chloramphenicol	28	1.8
Bacillus subtilis	**1**	12	0.2
	Chloramphenicol	35	2.5
Candida albicans	**1**	13.3	0.3
	Chlortrimazole	20	1.0
Trichophyton mentagrophytes	**1**	---[b]	0
	Chlortrimazole	36	2.6
Aspergillus niger	**1**	---	0
	Chlortrimazole	13	0.3

[a]Average of three trials, [b]No clearing zone

ACKNOWLEDGEMENT

The authors are grateful to Dr. John A. Rideout for conducting the NMR analyses at the School of Chemical and Biomedical Sciences, Central Queensland University, Rockhampton, Queensland, Australia. The antimicrobial tests were conducted at the University of the Philippines – Natural Sciences Research Institute.

REFERENCES

Adfa, M., Yoshimura, T., Komura, K., & Koketsu, M. (2010). Antitermite activities of coumarin derivatives and scopoletin from *Protium javanicum* Burm. F. *Journal of Chemical Ecology, 36*(7), 720-726.

Barua, R.K., & Barua, A.B. (1966). Oxidation of zeaxanthin, isolation and properties of 3-hydroxyretinene. *Biochemical Journal, 101*(1), 250-255.

Begum, T., Rahman, M. S., & Rashid, M. A. (2006). Phytochemical and biological investigations of *Phyllanthus reticulatus. Dhaka University Journal of Pharmaceutical Science, 5*(1-2), 21-23.

Crosby, D. G. (1961). Improved synthesis of scopoletin. *Journal of Organic Chemistry. 26*(4), 1215-1217.

Gupta, R. K., & Chandra, S. (1971). Chemical investigation of *Delonix regia* flowers. *Indian Journal of Pharmacy, 33*(4), 74-75.

Jahan, I., Rahman, M. S., Rahman, M. Z., Kaisar, M. A., Islam, M. S., Wahab, A., & Rashid, M. A. (2010). Chemical and biological investigations of *Delonix regia* (Bojer ex Hook.) Raf. *Acta Pharmaceutica, 60*(2), 207-215.

Juan, E. A., Rideout, J. A., & Ragasa, C. Y. (1997). Bioactive furanocoumarin derivatives from *Ficus pumila* (Moraceae). *Philippine Journal of Science, 126*(2), 143-153.

Lee, H. L., Lee, K. T., Yang, J. H., Baek, N. I., & Kim, D. K. (2004). Acetylcholinesterase inhibitors from the twigs of *Vaccinium oldhami* Miguel. *Archives of Pharmacal Research.* 27(1), 53-56.

Padolina, W.G., Yoshioka, H., Nakatani, N., Mabry, T. J., Monti, S. A., Davis, R. E., Cox, P. J., Sim, O. A., Watson, W. H., & Wu, I. B. (1974). Glaucolide-A and –B, new germacranolide type sesquiterpene lactones from *Vernonia* (compositae). *Tetrahedron, 30*(10), 1161-1170.

Pant, U.C., & Joshi, B. C. (1972). Chemical examination of *Delonix regia. Herba Polonica, 18*(2), 160-161.

Pandey, D.K., Chandra, H., & Tripathi, N. N. (1982). Volatile fungitoxic activity of some higher plants with special reference to that of *callistemon laceolatus* DC. *Journal of Phytopathology, 105*(2), 175-182.

Rahman, K. M., Nahar, K., Khan, M. G. U., & Hasan, C. M. (2007). Phytochemical and biological studies on *Naphelium longan. Boletin Latinoamericano y del Caribe de Plantes Medicinales y Aromaticas, 6*(3), 68-72.

Roy, S., & Sengupta, P. (1968). Chemical investigation of the bark of *Delonix regia. Journal of the Indian Chemical Society, 45*(5), 464-465.

Sharma, R. A., Chandrawat, P., Sharma, S., Sharma, D., Sharma, B., & Singh, D. (2010). Efficacy of *Delonix regia* rafin (syn. *Poinciana regia* bojer ex. hook) for potential antifungal activity. *The Bioscan, 5*(3), 441-444.

Siddiqui, B. S., Sattar, F. A., Ahmad, F., & Begum, S. (2007). Isolation and structural elucidation of chemical constituents from the fruits of *Morinda citrifolia* Linn. *Archives of Pharmacal Research, 30*(8), 919-923.

Su, J.-D., & Fan, C.-T. (2008). Antioxidative Activity and Mechanism of Isolated Components from Flowers of *Delonix regia. Food for Health in the Pacific Rim: 3rd International Conference of Food Science and Technology.* In J. R. Whitaker, N. F. Haard, C. F. Shoemaker & R. P. Singh. Food and Nutrition Press Inc. Trumbull, Connecticut, USA. pp. 243-52.